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Neurobehavioral Toxicity of Triethyltin in Rats as a Function of Age at Postnatal Exposure^{1,2}

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ABSTRACT: Triethyltin (TET) has been shown to be neurotoxic when injected on postnatal day (PND) 5. In the present experiment we examined the toxicity of a single exposure to TET at several postnatal ages. Rat pups were injected ip with 0 (saline), 1.5, 3.0 or 6.0 mg/kg TET bromide on PND 1, 5, 10 or 15. In agreement with our previous data, PND-5 exposure to 6 mg/kg TET produced behavioral toxicity and decreased adult brain weight. High dose pups were less successful in descending on a rope at 20 and 21 days of age, and were hyperactive in figure-eight mazes at 29-30 and 57-58 days of age. The spatial distribution of activity was also altered: photocell counts were increased primarily in the figure-eight area of the maze. The size of the milk bands was reduced in 6 mg/kg pups injected on either PND 1 or PND 5. Preweaning growth was decreased following all injection ages; this reduction was most pronounced for pups exposed to TET on PND 1 and PND 5. Mating behavior was disrupted in 6 mg/kg males irrespective of age at exposure. These data demonstrate a differential sensitivity to the toxicity of TET during postnatal life, with maximal susceptibility on PND 5.

Key words: Triethyltin, Postnatal Exposure, Developmental Toxicity

INTRODUCTION

Exposure of developing rat pups to TET on PND 5 produces persistent neurobehavioral toxicity (Harry and Tilson, 1981; Reiter *et al.*, 1981; Harry and Tilson, 1982; Ruppert *et al.*, 1983). This

exposure occurs during a period of rapid brain growth which, in rats, occurs during the first 3 wk of postnatal life. Major events of this stage of neural development are axonal and dendritic growth, synaptogenesis, proliferation of oligodendroglia and granule cells, mye-

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linogenesis, and neurochemical maturation (Davison and Dobbing, 1966; Dobbing, 1968; Ford, 1973; Cowan, 1979). Sexual differentiation of the nervous system, which is mediated by the action of gonadal hormones, also occurs within the first 2 wk of postnatal life in the rat (Goy and McEwen, 1980). Dobbing has proposed that this "brain growth spurt" is a time of special vulnerability to environmental disruption (Dobbing and Sands, 1971) because of the rapid growth and development of the nervous system which occurs at this time.

It has been repeatedly demonstrated that the type of damage and resulting behavioral deficits produced by a developmental neurotoxicant depend on the time of exposure as well as the mechanism of action of a toxicant (Rodier, 1980; Suzuki, 1980). TET is an interesting neurotoxicant to pursue in defining periods of vulnerability during the brain growth spurt because of its disruptive effects on myelin and energy metabolism (Cammer, 1980; Watanabe, 1980). In the present experiment, we evaluated the neurobehavioral toxicity resulting from a single exposure to TET at several postnatal ages (PND 1, 5, 10 or 15) which coincide with different stages of rapid brain growth in the rat (Gottlieb *et al.*, 1977). We compared the effects of TET at these exposure ages on mortality, body weight, brain weight and behavioral functions (rope descent, locomotor activity and the acoustic startle response) which had previously been shown to be sensitive to TET toxicity on PND 5 (Reiter *et al.*, 1981; Ruppert *et al.*, 1983).

METHODS

Animals

Pregnant CD rats (Charles River) were obtained 2 days after mating and individually housed in cages measuring 45 x 24 x 20 cm. One day after parturition (day of birth=day 0), pups were random-

ized and each dam was assigned 4 male and 4 female pups. At 21 days of age, animals were weaned and littermates were housed by sex in groups of 4 in the same size cages. Animals were maintained on a 12:12 hr light:dark cycle beginning at 0600 hr in an animal facility in which air temperature ($22^{\circ}\text{C}\pm 2$) and humidity ($50\%\pm 10$) were controlled. Except for male sexual behavior, behavioral testing was conducted during the diurnal portion of the cycle (0900 hr-1700 hr). For preweaning testing, all pups in a cage were tested sequentially to prevent repeated disturbance of litters, but testing was counterbalanced for age at exposure. For adult testing, animals within each test run were counterbalanced for sex, home cage, dose, and age at exposure. Food (Purina Lab Chow) and water were available *ad libitum* throughout the experiment.

Dosing

Rat pups were injected on PND 1, 5, 10 or 15 (N=10 litters/age at exposure). Each pup received a single intraperitoneal injection of either 0 (sterile, 0.9% saline), 1.5, 3.0 or 6.0 mg/kg triethyltin bromide (Alfa Products; Danvers, Ma.) calculated as the bromide. A within-litter design was used for dosing: 1 male and 1 female from each litter received each dose and, therefore, each litter contained all treatments. The volume of injection was 10 $\mu\text{l/g}$ of body weight for pups injected on PND 1 and 5, and 5 $\mu\text{l/g}$ of body weight for pups injected on PND 10 and 15.

Data Analysis

The design model for this experiment was a partial hierarchical four-way factorial with no within-cell replication. For all non-categorical data, analyses were performed using litter as the unit of analysis. Litter was assumed to be a random factor nested within PND exposure and crossed with treatment and sex.

Treatment, sex and PND exposure were considered fixed and completely crossed factors.

Univariate repeated measures techniques were used on BMDP (4V) to analyze pre- and post-weaning body weights, and maze activity (total, figure-eight area and blind-alley area). Age at exposure was the between factor and sex, treatment and age at testing were within factors. Univariate repeated measures were also used to analyze both the amplitude and latency of the acoustic startle response. Age at exposure was the between factor and sex, treatment and background level were within factors. Greenhouse-Geisser adjustments were used where appropriate with the overall level of significance set at $p < .005$. Separate ANOVA's were used to analyze organ weights. Age at exposure was the between factor and treatment was the within factor. The overall level of significance was set at $p < .01$. There was one missing value for preweaning body weight from PND 1 exposure which resulted in the deletion of the litter from the data. Also, due to equipment failure there were three missing values for the startle data (one each from PND 5, 10 and 15) which resulted in deletion of the entire litters from the analysis.

Due to the complexity of the experimental design, the individual animal was the unit of analysis of categorical data for milk-band ratings, rope descent and male sexual behavior. It was also difficult to adequately assess interactions of exposure age x treatment for categorical data. Nevertheless, statistical methods which were used for these measures are described below.

Body Weight

Animals were weighed at 1, 5, 10, 15, 20, 30, 44 and 58 days of age.

Milk Bands

As an estimate of milk consumption during the acute phase of TET intoxica-

tion, pups injected on PND 1 and PND 5 were examined for the presence and size of milk bands 24, 48, 72 and 96 hr after injection. At these ages, the contents of the stomach can be easily seen through the ventral abdominal wall. Pups injected on PND 10 and PND 15 were not rated because the thickening of skin over the abdomen prevented the viewing of milk bands. Milk bands were rated according to the following criteria: 0-no band visible; 1-small band visible on side of pup; 2-small band visible across pup's abdomen; 3-large band visible across pup's abdomen. The rater was not aware of the pup's treatment while performing the observation. Milk-band ratings were analyzed using the Kruskal-Wallis test (chi² approximation).

Descending on a Rope

On days 20 and 21, pups were tested for the ability to descend a 30 cm x 17 mm rope suspended above the home cage, using a procedure modified from Anderson and Altman (1972). Each pup was held by the nape of the neck and placed against the rope with its head pointing upward. Pups were allowed 180 sec to complete the test; 1 trial was given per day. Performance was scored as successful if the pup displayed either motor coordination or muscular strength by 1) descending the rope in either the head-up or head-down position, or 2) clinging to the rope for the duration of the test. Performance was scored as unsuccessful if the pup fell or slid from the rope during the test. The rater was not aware of the pup's treatment while performing the test. The proportion of successful animals/group was transformed (arcsin) and analyzed by repeated-measures ANOVA using test day as the within-subjects factor.

Motor Activity

Motor activity of individual animals was measured for 1 hr in a figure-eight

maze on days 29-30 and again on days 57-58. The maze (Reiter *et al.*, 1975) is a series of interconnected alleys (10 x 10 cm) converging on a central arena and covered with a removable, transparent acrylic-plastic top. Motor activity is detected by 8 phototransistor/photodiode pairs. Mazes (N=16) were housed in 2 sound-attenuated rooms maintained on the same light:dark cycle as the animal facility.

Startle Response

At approximately 70 days of age, latency (time interval between stimulus onset and the initiation of a response) and amplitude (maximum force exerted on the platform, corrected for the baseline) of the acoustic startle response were measured. Testing was conducted in 4 sound-attenuated chambers each containing a acrylic plastic-framed wire cage (7.6 x 7.6 x 23 cm) mounted on a load cell/force transducer assembly designed to measure vertical force. Two speakers were mounted on the ceiling of each chamber 30 cm above the test cage. One speaker presented an acoustic stimulus (13 kHz, 120 dB(A), 40 msec tone with a 2.5 msec rise time); the second delivered background white noise at each of 3 intensity levels (50, 65 and 80 dB(A)). These stimulus parameters were adapted from Hoffman and Searle (1965). Following a 10-min period of adaptation to the chamber at ambient noise levels, a total of 30 stimuli were presented at an inter-stimulus interval of 20 sec; 10 stimuli were presented at each of 3 background noise levels which were balanced across the session.

Male Sexual Behavior

Male rats received 3 weekly mating tests starting at approximately 90 days of age. Testing arenas were 37.5 x 47.5 x 20 cm acrylic-plastic cages with pine shavings covering the bottom. Testing was conducted during the nocturnal period (1900-2300 hr); the rater was unaware of

the males' treatment during the test. Males were allowed a 5-min adaptation period in the arenas before a sexually receptive female was introduced. Females were made receptive by 3 daily injections of 12 μ g estradiol benzoate followed by 500 μ g progesterone on the fourth day 4 hr before testing. Components of male sexual behavior (including frequency and latency measures for mounts, intromissions and ejaculation) were scored as described by Beach and Jordan (1956). Copulatory activity was observed until either 1) the male ejaculated, or 2) 20 min (wk 1 and 2) or 30 min (wk 3) had elapsed from the introduction of the female and no ejaculation had occurred. Since males were inexperienced at the start of testing, only data from the third week of testing were used for statistical analysis. The proportion of males ejaculating was analyzed using a 2 x 4 (performance x treatment) χ^2 test. The contingency coefficient, a measure of the strength of association, was also obtained. Other components of male sexual behavior were analyzed using MANOVA.

Organ Weights

Males were sacrificed after tests for mating behavior; body weight and wet weights for brain, thyroid, adrenals, seminal vesicles and testes plus epididymes were obtained.

RESULTS

Body Weight

Growth was reduced in dosed pups following all exposure ages (Fig. 1). However, these body weight decreases were most persistent following PND 1 and PND 5 exposure to TET. There was a three-way interaction of treatment x age x exposure for preweaning body weight [$F(13.18, 153.75) = 3.16$, $p < .0003$]; therefore, tests of simple main effects and simple interactions were performed. There

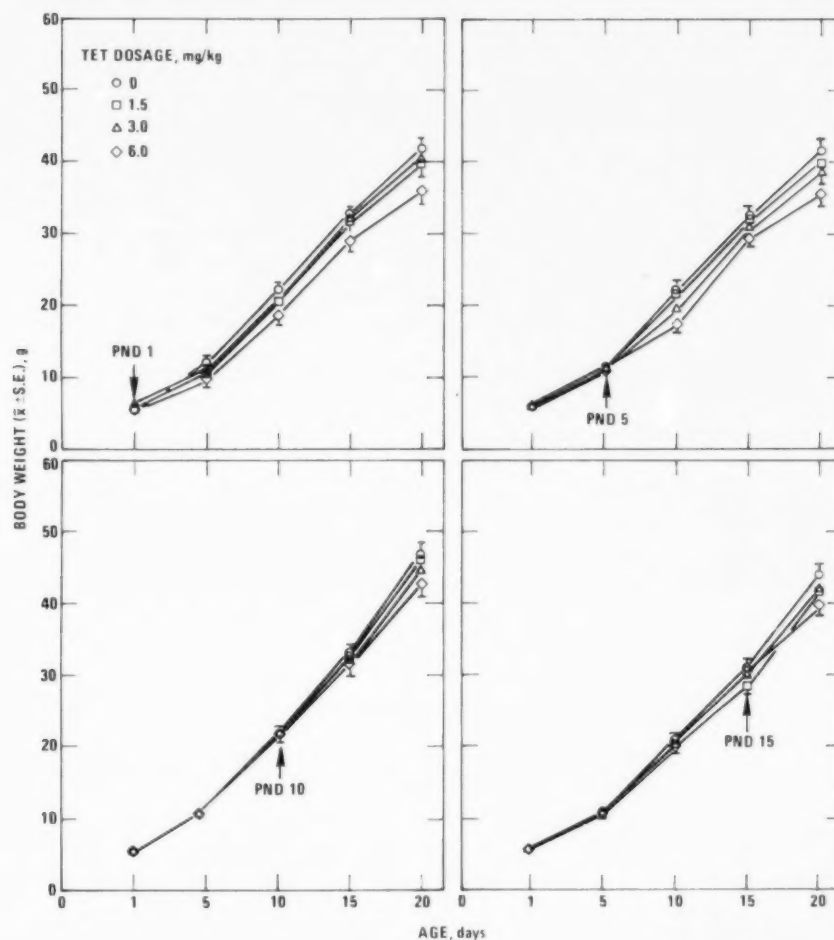


FIG. 1. Preweaning body weights ($\bar{x} \pm SE$) for control and TET-exposed rat pups. Arrow in each panel indicates when TET exposure occurred. For all days of exposure, TET produced a reduction in growth.

was a treatment x age interaction on PND 1, 5, 10 and 15 [$F(4.39, 153.75) = 11.17, 14.82, 6.81, 7.73$ respectively; all $p's < .00001$]. Tukey's (a) tests were used to make post-hoc comparisons. For PND 1 exposure, there was no treatment effect on day 1. On days 5, 10, 15 and 20 pups receiving 6 mg/kg TET weighed less than all other groups. For PND 5 exposure, there were no treatment effects on day 1 or day 5. On day 10, 15 and 20 pups receiving 6 mg/kg TET weighed less than

all other groups, and pups receiving 3 mg/kg TET weighed less than controls; pups receiving 1.5 mg/kg TET weighed less than controls on day 15 and 20. For PND 10 exposure, there were no treatment effects on days 1, 5 or 10. On days 15 and 20, pups receiving 6 mg/kg TET weighed less than all other groups, and on day 20 pups receiving 3 mg/kg TET weighed less than controls. For PND 15 exposure, there were no treatment effects on days 1, 5, 10 or 15. On day 20, pups

receiving all dosages of TET weighed less than controls, and pups receiving 6 mg/kg TET weighed less than all other groups.

Decreases in body weight were not persistent. At ages 30, 44 and 58 the usual sex effect on body weight was evident, with males being heavier than females, and body weight increased for both sexes with age. There were no effects of treatment or interaction with treatment.

Milk Bands

For pups injected on either PND 1 or PND 5, there was an effect of treatment on milk-band ratings 24 hr after dosing [$\chi^2(3)=27.53$, $p<.0001$]. Pups injected with 6 mg/kg TET on either PND 1 or PND 5 (data combined) had smaller milk bands than all other pups 24 hr after dosing but not at later times (Fig. 2).

Rope Descent

There was an improvement in performance for all rats from day 20 to 21 [$F(1,260)=28.96$, $p<.0001$], with no treatment $\times$ day of testing interaction [$F(3,260)=1.11$, $p<.3459$]. There was a treatment $\times$ exposure interaction [$F(9,73)=2.27$, $p<.0185$]; therefore, separate analyses were conducted for each exposure age. For PND-5 exposure only, there was an overall treatment effect [$F(3,73)=6.15$, $p<.0005$]. The percentage of successful animals was 87.5% for controls, 80.0% for 1.5 mg/kg, 65.0% for 3 mg/kg and 42.5% for 6 mg/kg TET. Animals injected with 6 mg/kg on PND 5 were less successful than controls in performing this task.

Figure-Eight Maze Activity

There was an interaction of treat-

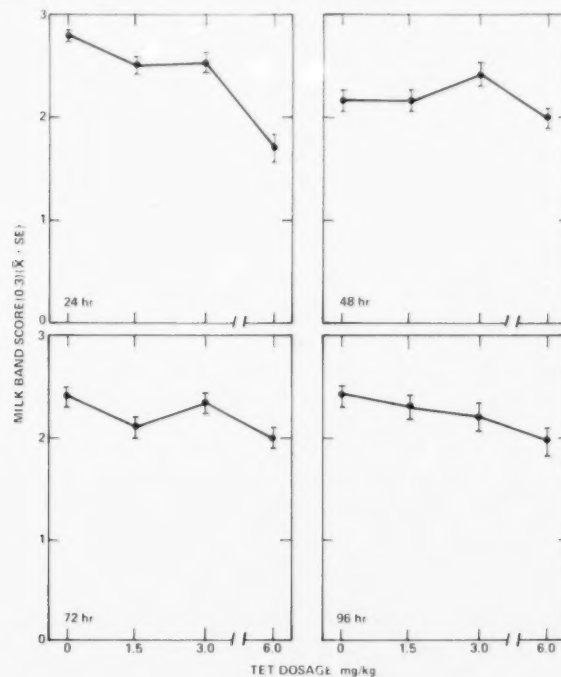


FIG. 2. Milk band ratings (0-3) measured 24-96 hr after injection for controls and pups exposed to TET on PND 1 or PND 5 ($\bar{X} \pm SE$). Pups receiving 6 mg/kg TET on either PND 1 or PND 5 (data combined) showed a reduction in the size of the milk bands 24 hr after dosing, indicating reduced milk consumption.

ment x exposure [$F(7.26,87.15)=10.72$, $p<.00001$] and sex x age [$F(1,36)=25.84$, $p<.00001$]; therefore, tests of simple main effects were performed. There was a treatment effect for exposure on PND 5 [$F(2.42,87.15)=41.78$, $p<.00001$], but not for other exposure ages. Tukey's (a) test showed that activity of animals receiving 6 mg/kg TET on PND 5 was increased compared to all other groups (Fig. 3). There was a sex effect on PND 57-58 only [$F(1,36)=29.83$, $p<.00001$], with females more active than males and an age effect for both males and females [$F(1,36)=28.22$ and 159.74 , respectively; $p's<.00001$], with activity on PND 57-58 higher than on PND 29-30.

For activity in the figure-eight area of the maze, there was a treatment x exposure interaction [$F(6.70,80.36)=14.33$, $p<.00001$]; therefore, simple main effect tests were performed. There was a treat-

ment effect for exposure on PND 5 [$F(2.23,80.36)=56.88$, $p<.00001$], but not for other exposure ages. Tukey's (a) test showed that motor activity of animals receiving 6 mg/kg TET on PND 5 was higher in the figure-eight area of the maze than all other groups (Fig. 4). There was also an age effect [$F(1,36)=457.76$, $p<.00001$] with older rats (PND 57-58) more active in the figure-eight area than younger rats (PND 29-30).

For activity in the blind-alley area of the maze, there was a sex x age interaction [$F(1,36)=29.36$, $p<.00001$]; therefore, simple main effect tests were performed. There was a sex effect on PND 57-58 only [$F(1,36)=160.14$, $p<.00001$], with females more active in the blind alleys than males, and an age effect for both males and females [$F(1,36)=142.72$, 12.06 respectively; $p's<.001$] with older rats (PND 57-58) less active in the blind-alley area

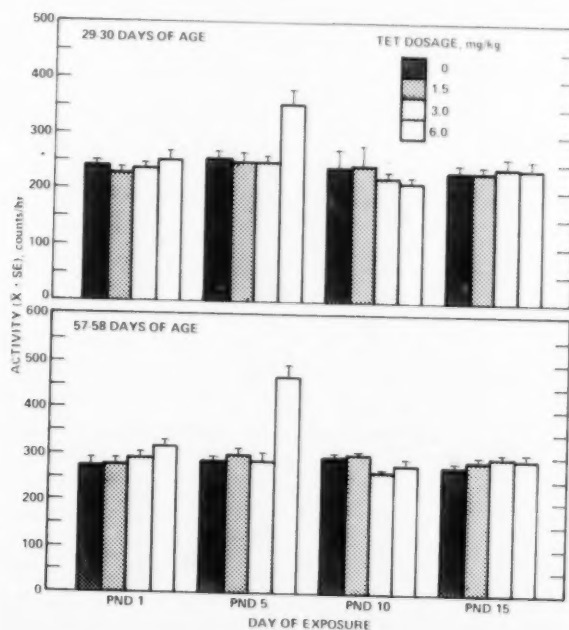


FIG. 3. Figure-eight maze activity for control and TET-exposed rats. Data are expressed as photocell interruptions ($\bar{X} \pm SE$) during a 1-hr test session. Exposure to 6 mg/kg TET on PND 5 produced an increase in motor activity at both times of testing.

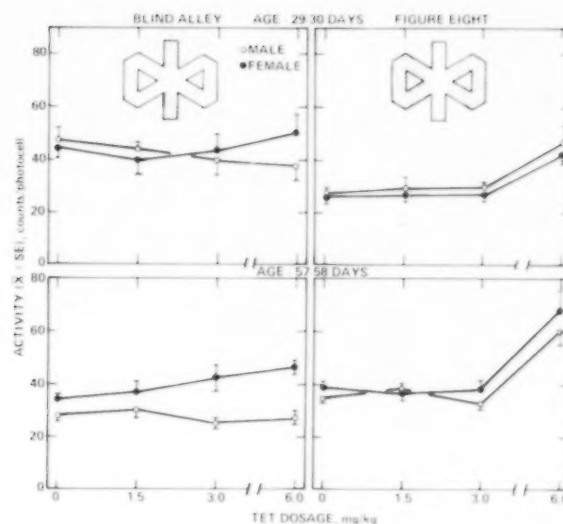


FIG. 4. Distribution of motor activity in figure-eight mazes for PND-5 control and TET-exposed animals. Data are presented as photocell counts ($\bar{X} \pm SE$) in either the blind alleys or figure-eight area of the maze. Young animals show a different pattern of activity than adults. Exposure to 6 mg/kg TET increased photocell counts primarily in the figure-eight area.

than younger rats (PND 29-30).

Acoustic Startle Response

For the amplitude of the startle response, there was a sex $\times$ background noise interaction [$F(1.52, 50.07) = 48.49$, $p < .00001$]; therefore, simple main effect tests were performed. There was an effect of background level for both males and females [$F(1, 33) = 152.29, 86.05$ respectively; $p's < .00001$], with the amplitude of the response increasing with increasing level of background noise. There was a sex effect at all background noise levels [$F(1, 33) = 82.20, 61.10, 33.31$ for 80, 65 and 50 dB respectively; $p's < .00001$], with the amplitude of the response greater in males than in females. For latency to onset of the response, there was an effect of background noise level [$F(1, 10, 36.45) = 8.25$, $p < .0055$], with latency of the response decreasing with increasing background noise levels. There were no effects of treatment or interactions with treatment.

Male Sexual Behavior

Since a preliminary test indicated no treatment $\times$ exposure age interaction for the proportion of males ejaculating [$\chi^2(9) = 1.65$, $p < .9959$], data were combined across all days of exposure. There was an effect of treatment on the proportion of males ejaculating during the third weekly mating test [$\chi^2(3) = 16.258$, $p < .001$]. The percent of males ejaculating was 92.3% for controls, 79.5% for 1.5 mg/kg, 94.9% for 3 mg/kg, and 64.1% for the 6 mg/kg dose group. Exposure to 6 mg/kg TET decreased the number of males ejaculating irrespective of age at exposure. The contingency coefficient, a measure of the strength of association, was 0.307. There were no treatment effects on latency or frequency for mounts or intromissions.

Organ Weights

For brain weight, there was an interaction of exposure $\times$ treatment

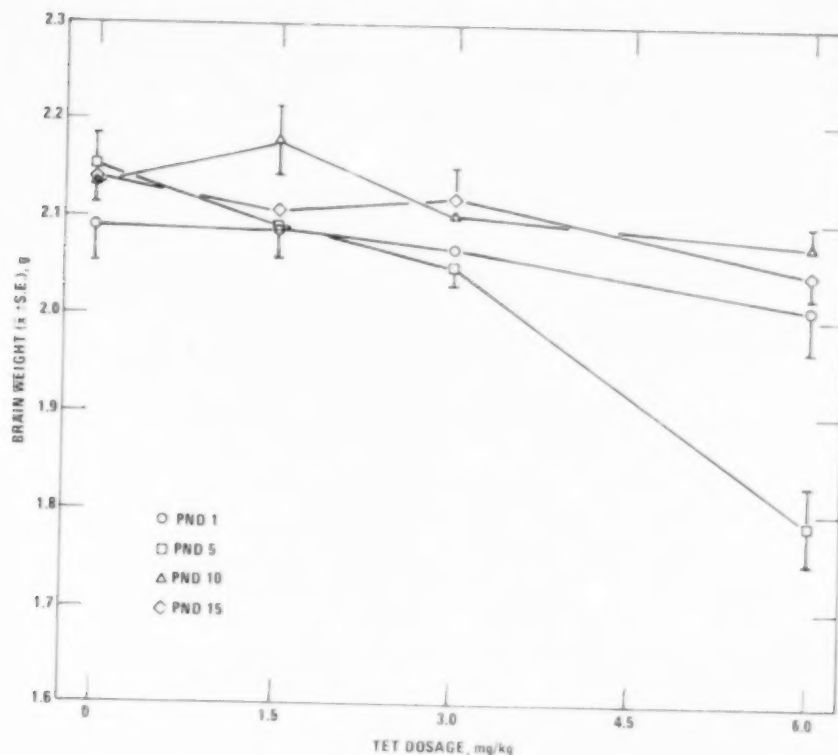


FIG. 5. Wet weight of the whole brain ($\bar{X} \pm SE$) for control and TET-exposed male rats. Exposure to 6 mg/kg TET on PND 5 only produced a decrease in adult brain weight.

[$F(8.20, 98.37) = 5.98$, $p < .00001$]; therefore, simple main effect tests were performed. There was a treatment effect for exposure on PND 5 [$F(2.73, 98.37) = 31.65$, $p < .00001$], but not for other exposure ages. Tukey's (a) test showed that brain weight of animals receiving 6 mg/kg TET on PND 5 was reduced compared to all other groups (Fig. 5). For seminal vesicle weights, there was a treatment x exposure interaction [$F(7.81, 93.75) = 2.82$, $p < .0081$]; therefore, simple main effect tests were performed. There was a treatment effect for exposure on PND 5 [$F(2.60, 93.75) = 5.22$, $p < .0035$], but not for other exposure ages. Tukey's (a) test showed that seminal vesicle weights of rats receiving 6 mg/kg TET were larger than those of rats receiving 1.5 mg/kg TET. There were no

effects on thyroid, adrenal or testes and epididymes weights.

DISCUSSION

The toxicity produced by TET during the preweaning period in the rat is dependent on the day of exposure. TET had its maximal effects as a developmental neurotoxicant following exposure on PND 5. Behavioral changes following acute exposure to TET only on PND 5 include preweaning deficits in rope descent performance and adult hyperactivity. In animals exposed to TET as adults, toxicity is reversible following termination of exposure (Magee *et al.*, 1957; Reiter *et al.*, 1980; Squibb *et al.*, 1980;

Tilson and Burne, 1981). Since the type of disorder produced by toxicants can differ as a function of the time during development when exposure occurs (Rodier, 1980; Suzuki, 1980), other behavioral tests might detect disruption of function specific to exposure at other developmental ages. However, brain weight was decreased only in animals exposed to TET on PND 5. These behavioral and brain weight data indicate that PND 5 is a time of special vulnerability to the toxicity of TET.

Milk bands were reduced when evaluated 24 hr after dosing with TET on PND 1 and PND 5; this effect did not persist beyond 24 hr. Milk bands were reduced to a similar extent following 2 hr of deprivation, and were correlated with reduced stomach weight (Dean, 1983), indicating decreased milk consumption. Therefore, a decrease in the size of milk bands reflects a transient lack of nutrition during the acute phase of TET intoxication. Prewaning growth retardation is a reliable indicator of TET toxicity (Reiter *et al.*, 1981). Since milk bands were reduced only 24 hr after dosing and not at later times, decreased preweaning body weight may reflect an additional metabolic effect of TET. Milk bands in pups injected at later ages could not be evaluated because of thickening of the skin over the abdomen and the development of hair. However, treatment effects on body weight were most pronounced for pups injected on PND 1 and PND 5. Since body weight was transiently reduced in pups injected at older ages (PND 10 and PND 15), food intake may have been reduced in these older pups as well. Food intake, and to a lesser extent water intake is reduced in adult animals receiving TET (Squibb *et al.*, 1980; DeHaven *et al.*, 1982). Adult animals are also hypoactive 2 hr after TET injection (Reiter *et al.*, 1980). Since activity level influences suckling in young rat pups (Blass *et al.*, 1979), decreased milk intake in rat pups

exposed to TET could be a result of decreased activity. However, serotonergic and cholinergic antagonists also suppress suckling behavior in young rat pups (Caza and Spear, 1982; Spear and Ristine, 1982). Acute administration of TET to adult rats depletes several neurotransmitters including serotonin (Moore and Brody, 1961; Robinson, 1969). Therefore, reduced milk bands and the resultant preweaning growth reduction may be specific effects of TET.

Hyperactivity was produced by TET injected on PND 5 only. PND-5 TET also altered the spatial distribution of activity in developing and adult animals. Young (day 30) control rats of both sexes distribute their activity in a figure-eight maze unequally: photocell counts are higher in the blind alleys than in the figure-eight portion of the maze. As adults, overall activity increases, but only in the figure-eight area of the maze; photocell counts in the blind alleys actually decrease. At both ages of testing, hyperactivity produced by 6 mg/kg TET was primarily due to an increase in photocell counts in the figure-eight area of the maze. Therefore, PND 5 TET animals remained hyperactive while the distribution of activity in control animals changed from a juvenile to an adult pattern. In addition to TET not producing hyperactivity following exposure at ages other than PND 5, the pattern of photocell interruptions was consistent between these groups.

Other behaviors previously shown to be disrupted by PND 5 TET were not affected by dosing at other postnatal ages. Pups exposed to 6 mg/kg TET on PND 5 were less successful in descending on a rope; this deficit persists with repeated testing from days 15-20 (Reiter *et al.*, 1981). Although rope descent tests motor coordination (Altman and Sudarshan, 1975), neuromuscular weakness could produce deficits in this task. Neuromuscular weakness results from adult exposure to TET (Barnes and Stoner,

1959). However, performance of pups exposed to TET on PND 5 was normal on several tests of neuromotor function—negative geotropism, placing reaction, head elevation and ascending on a wire mesh (Reiter *et al.*, 1981). Also, Harry and Tilson (1981) found no effects on grip strength or negative geotaxis at 21, 28, 60 or 90 days of age. In this experiment, PND-15 TET animals showed no impairment of function when tested for rope descent only 5 days after dosing.

The results of the present study do not agree with previous findings (Harry and Tilson, 1981; Ruppert *et al.*, 1983) that exposure to TET on PND 5 decreased the amplitude of the acoustic startle response. This discrepancy was likely due to the use of the litter as the unit of analysis and a more conservative criterion of significance in the present study ($p < .005$), as the data showed the same trends we had reported previously (Ruppert *et al.*, 1983). Consistent relationships were found for sensitization by background noise: the amplitude of the response increased and latency decreased with increasing levels of background noise. Neither sensitization, amplitude nor latency of the response was affected by postnatal exposure to TET at any age in the present experiment.

Sexual behavior was altered in high-dose males exposed to TET at all postnatal ages. A significant percentage of 6 mg/kg TET males failed to ejaculate after repeated pairing with a female in a restricted (30 min) mating test. Neither seminal vesicle nor testis + epididymis weight was decreased by treatment, suggesting that this behavioral deficit was not due to a direct toxic effect on the gonads. Hexachlorophene, whose toxicity resembles that of TET (Towfighi, 1980), produces a similar deficit in mating behavior when administered from day 1-8 postnatally (Gellert *et al.*, 1978). Following a dosage which produced 25% mortality, all males mounted and intromitted, but only 20-

40% ejaculated; this deficit was seen during fertility tests as well as during a restricted mating session. There were no alterations in serum testosterone levels or testes weight. Therefore, deficits in sexual behavior seen in the present study may indicate impaired fertility in TET males as well. Since effects of TET were similar following injection at all postnatal days, toxicant exposure might be disrupting processes involved in sexual differentiation which can extend through the first 2 wk of postnatal life in the rat (Gorski *et al.*, 1977; Goy and McEwen, 1980). Alternatively, TET may produce the same mating deficits when given to adult males because of its depletion of hypothalamic neurotransmitters (Cook, 1983). Further studies are needed to explore these possibilities.

PND-5 TET produces persistent effects on myelin (Wender *et al.*, 1974; O'Callaghan *et al.*, 1983; Padilla *et al.*, 1982), neuronal development (Veronesi *et al.*, 1982), and other neurochemical processes (Harry and Tilson, 1982) which may be specific to that age of development. TET is neurotoxic when given at postnatal ages other than PND 5. Suzuki (1971) reported that hemorrhage and neuronal necrosis at lethal dosages of TET were less severe in rat pups injected on PND 8 than in newborns. Intramyelinic vacuolation was seen in mouse pups following exposure on PND 5, 10, 15 or 20, but few vacuoles remained 1-2 wk after dosing (Watanabe, 1977). For other heavy metals, such as cadmium, the pathology produced by single dosages changes substantially between PND 1 and PND 15 (Gabbiani *et al.*, 1967; Webster and Valois, 1981). These differences are predictable, given the complex development encompassed within the "brain growth spurt". It remains to be determined why TET produces persistent and specific changes in behavior and reduction in brain weight following exposure on PND 5.

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